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SIX MUTATIONS AFFECTING COAT COLOR IN RANCH-BRED MINK [1]

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TWELVE mutant genes affecting coat color in the ranch-bred mink (*Mustela vison,* Peale and Beauvois) are now recognized. The well-known and commercially valuable color phases resulting from the substitution of six of these for their non-mutant alleles have been described by Smith *et al.* (1941), Shackelford (1941) and Castle and Moore (1946). The last mentioned authors have called attention to a few color phases resulting from combinations of certain of the mutant genes.

Smith *et al.* have proposed *p* as the symbol for the mutant gene for platinum, *b* for pastel and *S* for black cross. Castle and Moore have assigned c^a to the gene for near albinism, *ip* for imperial platinum and *F* for blufrost. Although Castle (1946) later suggested *S* rather than *F* as the correct symbolization of the gene for blufrost by the rule of priority, by this same rule *F* should be considered the proper symbol because of the following circumstances: The color phase known at present as black cross was originally designated silver by Smith *et al.* and the mutant gene symbol-

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ized by *S:* Castle incorrectly states that silver "came to be known later by the popular name silver sable, and, more recently, blufrost." Therefore, since Smith *et al.* symbolized the gene for black cross and not the one for blufrost, Castle and Moore's original use of *F* is valid.

The known mutant genes affecting coat color in American ranch-bred mink and foxes were enumerated and briefly discussed in a review paper given before the Eighth International Congress of Genetics by this writer. The six genes in the mink which had already been symbolized by other workers as indicated above were included in the paper along with the six to be considered here.

It is not uncommon to encounter considerable confusion when working with mutant color phases in the mink. The same basic genotype may have been known at various times since its recognition by four or five popular names. On the other hand, phenotypically similar but genetically different color phases have often borne the same name at some time in their development. In common with some other mammals, several of the mutant color phases in the mink seem to have "cropped up" at geographically widely separated places. These conditions make it difficult if not impossible to accredit correctly any given person as the "originator" of a certain color phase.

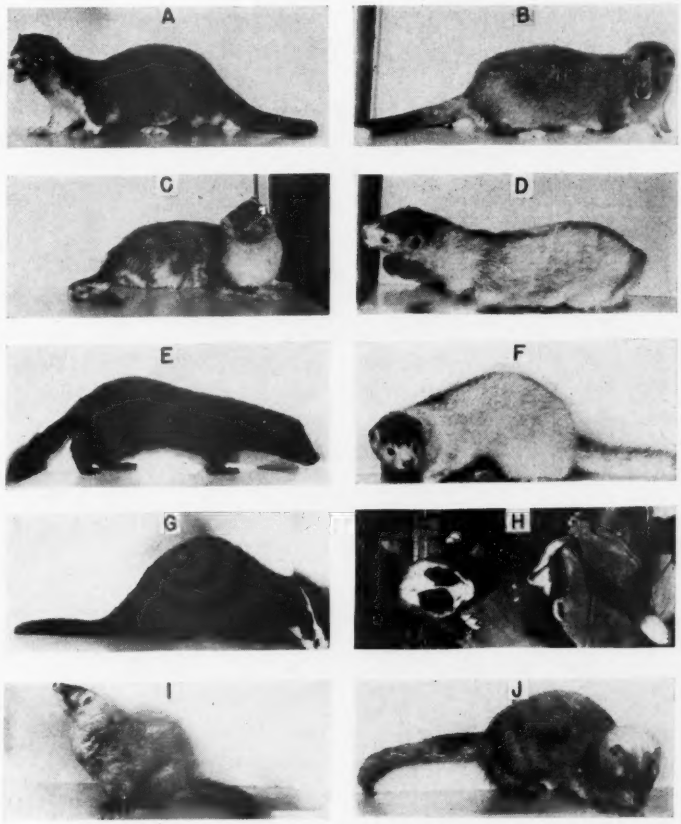
The treatment of the color phases to be considered in this paper will be based on the breeding results from our experimental herd in so far as possible, liberally supplemented with records taken from commercial breeders either for additional data or to supply data which are entirely lacking at this time. Speculation as to the probable value of any of these mutant genes for producing "new" color phases of commercial value will be left to those better qualified to consider such matters.

Dominant Color Phases

Royal Silver

Choker, royal silvered and simply royal silver are some of the names used to designate a pattern that has been

known to breeders for several years as one of the "*dominant*" color phases. It differs from the ordinary dark mink (wild type) mainly in the extension of the ventral white spotting to include the entire chin, throat, breast, belly and sometimes the feet and tail tip (Figure 1, A and B). Often



EXPLANATION OF PLATE

FIG. 1. Some color phases of the ranch-bred mink. A. Royal Silver (s^Rs). B. Glacierblu (s^Rspp). C–D. Blucross (Ffs^Rs). E. Ebony (*Ebeb*). F. Black cross heterozygous for Royal Silver (Ss^R). G–H. Colmira (*Cmcm*). I–J. Goofus (*oo*).

completely white guard hairs are scattered over the dorsal parts and the ventral white regularly extends up to the base of the ears. As with all the color phases in the mink, especially patterns, royal silvers differ considerably in appearance one from the other presumably as a result of modifying factors. Some of the least pigmented individuals in the royal silver group might occasionally be confused with the most heavily pigmented members of the black cross color phase.

Some breeders believe a darkening of the color in the guard hairs not affected by the white spotting to be regularly associated with this color phase. The breeding results in our herd, however, indicate that the darker color is dependent upon the residual inheritance rather than the mutant gene for the royal silver pattern.

Royal silver was introduced into our herd via two males presented by Dr. S. S. Osborn, of Waterville, Minnesota, in 1946. The pedigree of these males was unknown, but breeding results indicate that they are homozygous for the gene (s^R) responsible for the royal silver pattern (Table I A). Seven royal silver (s^Rs) offspring of these males in matings to non-royal silver (ss) produced 11 litters with a total of 23 royal silver to 21 non-royal silver young (Table I B). The two original royal silver males (2.1 and 2.2 in Table I A) are phenotypically similar to their offspring which are known by pedigree and breeding results to be heterozygous for s^R.

A female kit that in all respects is prenotypically identical with the royal silver color phase was born of natural dark (wild type) parents in 1945 on the Fromm Brothers, Nieman and Company ranch at Thiensville, Wisconsin. Since the royal silver had not previously been observed on this ranch, it seems probable the color phase of the kit resulted from a mutation at the s locus. The breeding history of this female and her royal silver offspring, both male and female, is similar to that of the royal silvers (s^Rs) in our herd.

TABLE I

BREEDING RESULTS OF THE ROYAL SILVER COLOR PHASE

	Type Mating	Offspring: Royal Silver ♂	Royal Silver ♀	Non-Royal Silver ♂	Non-Royal Silver ♀	Number litters	Average litter size
	Royal Silver ♂ 2.2 × Non-Royal Silver ♀♀	24	16	0	0	9	4.4
A	Total	40		0			
	Royal Silver ♂ 2.1 × Non-Royal Silver ♀♀	13	12	0	0	6	4.2
	Total	25		0			
	Royal Silver ♂♂ × Non-Royal Silver ♀♀	6	4	7	3	6	3.3
B	Non-Royal Silver ♂♂ × Royal Silver ♀♀	8	5	5	6	5	4.8
	Observed	23		21			
	Expected (1:1)	22		22			

		Black Cross ♂	Black Cross ♀	Royal Silver ♂	Royal Silver ♀	Dark ♂	Dark ♀		
C	Black Cross ♂ × Royal Silver ♀♀	4	5	1	1	1	0	4	3.0
	Observed	9		2		1			
	Expected (2:1:1)	6		3		3			
	Black Cross ♂ 28 × Dark (Wild Type) ♀♀	3	3	2		0	0	4	3.5
D	Dark (Wild Type) ♂♂ × Black Cross ♀ 109	1	1	3	1	0	0	2	3.0
	Observed	8		12		0			
	Expected (1:1)	10		10		0			
	(2:1:1)	10		5		5			

		Blucross	Royal Silver	Blufrost	Dark		
	Dark (Wild Type) ♂♂ × Blucross ♀♀	16	10	11	15	12	4.3
	Expected (1:1:1:1)	13.25	13.25	13.25	13.25		

		♂	♀	♂	♀	♂	♀	♂	♀		
E *	Blucross ♂ 2 × Dark (Wild Type) ♀♀	2	0	0	1	2	0	1	0	2	3.0
	Dark (Wild Type) ♂♂ × Blucross ♀ 4	0	1	1	0	0	1	0	1	2	2.0
	Observed	3		2		3		2			
	Expected (1:1:1:1)	2.5		2.5		2.5		2.5			

* Data obtained from Dr. S. S. Osborn, Waterville, Minnesota.

Castle and Moore (1946) reported "an imperfectly tested dominant mutation" which produced a color phase they

have termed "Blackout." This may be the same mutant gene (s^R) with which we are dealing here. The picture of the heterozygous individual shown in their paper is a good representation of either the heterozygous or homozygous royal silver mink in our herd, although none of our royal silvers have approached the appearance of the one they have called a "Homozygous Blackout individual." Further, these workers have suggested that "Possibly it (Blackout) is an allele of Kohinur." As will be seen from the discussion to follow, the evidence from our breeding experiments supports the hypothesis that the mutant genes for royal silver (s^R) and black cross (S) are alleles.

That the black cross color phase expresses itself to the exclusion of the royal silver was indicated by the results of black cross $\times$ royal silver matings in 1947 (Table I C). Approximately twice as many black cross kits occurred as would be expected unless about one-half of them were also heterozygous for the gene for royal silver. Two out of three of the phenotypically black cross individuals (one shown in Figure 1 F) from this type mating which were test mated to darks (wild type) produced among their offspring typical royal silver kits, thus proving them to be heterozygous for the gene for the royal silver pattern. The breeding results of these two black cross mink for 1948 and 1949 are shown in Table I D. Together they have produced 8 black cross and 12 royal silver kits when mated to dark (wild type) mink.

One kit representing the dark class would have been sufficient to demonstrate that S and s^R are not alleles. Or, if a black cross individual from this type mating produced royal silver offspring when mated to wild type, this would also demonstrate non-allelism; one such black cross has been tested, and has thrown black cross and dark kits only. If it is assumed that S and s^R are non-allelic, then the expected ratio in Table I D would be 10 black cross to 5 royal silver to 5 dark kits. The observed ratio of 8 black cross to 12 royal silvers is a very poor fit to the expected 2:1:1 ratio ($X^2 = 15.2$, $P = .0005$). On the assumption that S and s^R are alleles, the observed ratio is a good fit to the

expected 1 : 1. Thus the evidence indicates that S and s^R form an allelic series, with dominance in the direction $S \rightarrow s^R \rightarrow s$.

Osborn (1946) has mentioned a color phase which he calls blucross from blufrost (*Ffss*) × royal silver (*ffs*^{R}*s*) matings. Although some individuals of this color phase (*Ffs*^{R}*s*) differ slightly in appearance from the ordinary black cross (*Ss*), most of them are so similar to black cross that they would be classed among the commonly observed variations of black cross by those uninformed as to their genotype (compare C and D with F in Figure 1). Confusion has arisen as a result of using this name for the genotype *Ffs*^{R}*s*. Blucross as a color phase name is more often applied to the genotype *Sspp*, that is, the " black cross pattern on platinum " combination. Osborn's usage of blucross for the genotype *Ffs*^{R}*s* will be followed in this paper.

Twelve litters from blucross (*Ffs*^{R}*s*) × dark (*ffss*) matings as observed and recorded on the Osborn ranch in June, 1946, are shown in Table I E. The presence of either the blucross (*Ffs*^{R}*s*) class or the dark class (*ffss*) is sufficient evidence that s^R and F are not alleles. Further, the four classes appeared in approximately equal numbers, indicating that these two genes are borne on different chromosomes ($X^2 = 2.0$, $P > .55$), or if on the same chromosome, are not closely linked.

A blucross male and female were produced from a blufrost × royal silver mating in our herd in 1946. The two blucrosses are shown in C and D of Figure 1. The breeding results of these two mink in the 1947 and 1948 seasons are shown in Table I E, confirming the data collected on the Osborn ranch in 1946.

The color phases resulting from s^R in combination with *p*, *b* and *Cm* have been observed. Mink of the genotype *s*^{R}*spp* are royal silver patterned platinums sometimes called glacierblu by breeders (Figure 1 B). *s*^{R}*sbb* is a pastel with the royal silver pattern. In a mink of the genotype *s*^{R}*sCmcm*, the separate effects of both s^R and *Cm* are evident with no indication of interaction between them.

Colmira

Osborn (1946) has called attention to another type of white spotting in the mink and has designated it colmira (Figure 1 G and H). In common with royal silver, colmira has been known to mink breeders for some years as one of the several "dominant" color phases. So far as is known the gene (*Cm*) for colmira spotting has been observed only in the heterozygous condition, presumably because of a lack of matings designed to test its affects when homozygous.

Like the royal silver pattern, the ventral white spotting in the colmira color phase includes the chin, throat, breast, belly, sometimes the feet and tail tip (Figure 1 H), and often completely white guard hairs are sprinkled over the dorsal parts. Unlike royal silver or any of the other white spotting patterns in the mink, colmira spotting is rarely symmetrical on the head and neck. The white in these areas may range from a narrow blaze up the nose and forehead (Figure 1 G), to a large blaze joining with a complete collar about the neck including considerable portions of the shoulders; in the latter extreme, the only pigmented areas about the face may be a colored spot around each ear (Figure 1 H) rarely of the same size and sometimes around only one ear. Colmiras with much white about the face and head are occasionally called "panda" by mink breeders. Along with the asymmetry of the white spotting, the underfur in the colmira is characteristically lighter in color than in the natural dark. The brief description and picture in Castle and Moore's 1946 paper indicates that their "Baldy" is at least phenotypically similar to the color phase here termed colmira.

Colmira matings in our herd are limited to a single male (Figure 1 H) presented by Dr. Osborn in 1947, and two of his male offspring. This male, M38, proved to be heterozygous for *Cm* as shown in Table II. Four litters produced by his colmira offspring in matings to non-colmira mink gave 7 colmira to 10 non-colmira kits, a close approximation to the expected 1:1 ratio ($X^2 = .52$, $P = .50$).

TABLE II

COLMIRA × NON-COLMIRA MATINGS

	Type Mating	Offspring				Number litters	Average litter size
		Colmira		Non-Colmira			
		♂	♀	♂	♀		
A	Colmira ♂ M38 × Non-Colmira ♀♀	9	3	2	1	4	3.8
B	Colmira ♂♂ × Non-Colmira ♀♀	3	4	4	6	4	4.3
	Total	19		13			
	Expected (1:1)	16		16			

A mating of colmira (*Cmcmff*) × blufrost (*cmcmFf*) resulted in a litter containing 3 blufrost-colmira kits, 2 blufrosts and 1 colmira. From the appearance of the three mink showing the effects of both *Cm* and *F*, these two mutant genes appear to exert their separate effects with no indication of interaction in the combination *CmcmFf*. A blufrost-colmira (*CmcmFf*) male from this litter mated to dark (*cmcmff*) females has produced two litters which total 1 dark, 1 blufrost and 2 colmira kits. The appearance of the single dark individual is sufficient evidence that *Cm* and *F* are not alleles. A male mink from a black cross (*Sscmcm*) × colmira (*ssCmcm*) mating which showed the effects of both *S* and *Cm* was obtained from Dr. Osborn in 1948. This colmira-black cross male (*SsCmcm*) gave two litters when mated to dark (wild type) females; one contained 2 colmira, 2 black cross and 1 dark, and the other was composed of 1 colmira, 1 black cross and 1 dark. The presence of the dark kits (*sscmcm*) indicated that *S* and *Cm* are non-allelic.

Cmcmbb is the most commonly observed combination of colmira with any of the other color phases and is known among breeders as the "red-eyed blue taupe" color phase. This combination produces a white-spotted pastel with light underfur, no individuals with other than "red" eyes (darker than the eyes of the albino) having been observed. Mink of the genotype *Cmcmpp* are colmira patterned platinums. The $s^{g}sCmcm$ genotype has already been considered.

The original colmira male M38 and all of his 19 direct or

indirect colmira descendants show a remarkable lack of sensitivity to disturbances when sleeping. This does not appear to be due to an inability to hear, since the colmira, when awake, responds to ordinary sounds as well as other mink do. The degree to which colmira mink can resist disturbances and remain asleep is best demonstrated by approaching a nest box in which a mixed litter is sleeping. A touch of the nest box or at most raising the top is sufficient to send all non-colmira kits scurrying, but their litter mate colmiras usually remain asleep and can be touched or even gently picked up and replaced without awakening. Once awake, however, the colmira is as active as other mink.

Another behavior pattern associated with the colmira color phase is an apparent lack of coordination which in certain of its aspects is somewhat suggestive of "screw neck" (Shackelford and Cole, 1947) in the brown-eyed pastel, but in other aspects is quite different. Nearly all colmira patterned individuals throw the head over the back at times as do some pastels, but unlike the pastels they do not tilt the head to either one side or the other. A few colmira individuals run in small circles, but again unlike circling pastels do not necessarily have to be disturbed in order to exhibit circling. In general colmiras seem to be less upset by disturbances than do pastels, and sometimes actually appear to enjoy practicing the circling antics.

Three of these 20 colmiras, all males, are subject to convulsive seizures. Unusual noises or disturbances do not seem to be involved in precipitating the seizure. The affected mink will be running about in its cage like a normal individual, then suddenly fall prostrate, and a period of general twitching and jerking with foaming at the mouth follows and may last 20 to 30 minutes. When the mink has recovered it appears to be quite normal and shows no ill effects of the experience.

Ebony

A coat color that has been recognized by breeders for

some years as belonging to the "dominant" group is called ebony or ebonyblu, and in contrast to the other color phases seems never to have been known by other names. The underfur of the ebony is considerably lighter than that of the natural dark mink, ranging from near white to blue gray. The color of the guard hairs and the ventral white spotting remain the same as in the natural dark. In a segregating litter, the very young ebony kits can usually be distinguished from their non-ebony litter mates by the lighter appearance, particularly on the nose, face and feet.

Matings involving the ebony color phase in our herd are limited to two individuals, a male and a female, which are heterozygous for the gene (*Eb*) for ebony since they have produced both ebony and non-ebony kits when mated to non-ebony mink. The complete breeding data for these two mink can not be evaluated at this writing because of our inability to classify phenotypically some individuals having other dominant genes which may be obscuring the effects of *Eb*.

TABLE III *

BREEDING RESULTS OF THE EBONY COLOR PHASE

	Type Mating	Offspring				Number litters	Average litter size
		Ebony	Non-Ebony				
A	Ebony ♂♂ & ♀♀ × Non-Ebony	87	102			46	4.1
	Expected (1:1)	94.5	94.5				
B	Ebony (EbebBb) ♂♂ & ♀♀ × Pastel (ebebbb)	Ebony (EbebBb) 22	Dark (ebebBb) 14	Palomino (Ebebbb) 7	Pastel (ebebbb) 37	15	5.3
	Expected (1:1:1:1)	20	20	20	20		

* Data obtained from Christensen Brothers Minkery, Cambridge, Wisconsin.

The data shown in Table III were taken from the 1948 breeding records of the Christensen Brothers Minkery, Cambridge, Wisconsin. Ebony (derived from ebony × natural dark matings) × natural dark matings gave 102 natural dark to 87 ebony kits, a ratio which does not differ significantly from 1:1 ($X^2 = 1.2$, $P > .27$).

Within certain limits the most desirable pelt in any self-

colored mink is the one showing greatest contrast between the guard hair and the underfur. In order to enhance the "two tone" effect of the pastel (*bb*), several breeders have made the *Ebebbb* combination producing a color phase called by some palomino. This combination has been achieved by making *EbebBB* × *ebebbb* matings, followed by *EbebBb* × *ebebbb* matings. Tale III B shows the results of the latter type mating in 1948 on the Christensen Brothers' ranch.

On the assumption that *Eb* and *b* are on different chromosomes the four expected color phases should approximate a 1:1:1:1 ratio. The observed 22 ebony (*EbebBb*) : 14 dark (*ebebBb*) : 7 palomino (*Ebebbb*) : 37 pastel (*ebebbb*) is a very poor fit to the expected ratio ($X^2 = 20.0$, $P = .0002$). If it be assumed that *Eb* and *b* are on the same chromosome, then *Eb* and *b* are located approximately 26 crossover units apart since the non-crossover classes (ebony and pastel) comprise 74 per cent. of the total from this type mating and the crossover classes (palomino and dark) 26 per cent. So far as is known these data furnish the first evidence of linkage in the mink.

Blufrost (*Ffebeb*) × ebony (*ffEbeb*) matings have been made in our experimental herd as well as on a number of commercial ranches. *Eb* and *F* do not appear to interact in the combination *EbebFf*. Mink of this genotype closely resemble the blufrost color phase, but can usually be distinguished from it by the lighter tone of the underfur along with a lighter brown patch over each eye somewhat resembling an eyebrow, and the light brown margins of the ears.

Several breeders have attempted to obtain the ebony-platinum combination (*Ebebpp*). Either this genotype has not been produced, or its phenotype is similar to platinum since only ebony, dark and platinum kits have been observed among more than 150 offspring from *EbebPp* × *ebebpp* matings. The mink homozygous for *Eb* has either not been produced or else not recognized.

Recessive Color Phases

Aleutian

Langenfeld (1947) has given an account of the occurrence and early breeding history of a color phase originally called Waris blue, but now most commonly known as aleutian. This color phase results from a third mutation that dilutes the natural dark coat color to a blue gray. Although the overall appearance of the aleutian suggests the platinum (*pp*) and imperial platinum (*ipip*) phases which are phenotypically similar, it is darker and more nearly approaches a steel gray color. The smoother or softer tone of the aleutian in comparison with platinum or imperial platinum probably is a result of the distribution of the pigment granules in the hair; in the platinum (Shackelford, 1948) the dark brown pigment granules are for the most part in large clumps, while in the aleutian what appears to be a reduced quantity of pigment is evenly distributed within the hair. The eyes of some aleutians are red when seen at certain angles.

All breeders of this color phase are agreed that the gene (*al*) for aleutian is recessive to its non-mutant allele since dark (wild type) offspring only are produced from aleutian $\times$ dark matings, back cross matings of *Alal* $\times$ *alal* give aleutian and dark kits in approximately equal numbers, and aleutian $\times$ aleutian matings produce only aleutian kits. We have had only two matings involving the aleutian color phase in our herd. In the 1948 breeding season Associated Fur Farms mated two of our dark (wild type) females to aleutian males. The 13 kits from these matings were all dark (Table IV A). Test matings in 1947 and 1948 at Associated Fur Farms of aleutian (*alalPP*) $\times$ platinum (*AlAlpp*) and aleutian (*alalIpIp*) $\times$ imperial platinum (*AlAlipip*) gave only wild type offspring as would be expected if *al* represented a mutation at a different locus from *p* and *ip* (Table IV B and C).

Several color phases resulting from the combination of *al* with other mutant genes are popular among breeders. The

TABLE IV

BREEDING RESULTS OF THE ALEUTIAN COLOR PHASE

	Type Mating	Offspring: Aleutian ♂	Offspring: Aleutian ♀	Offspring: Dark ♂	Offspring: Dark ♀	Number litters	Average litter size
A	Aleutian ♂♂ × Dark ♀♀	0	0	10	3	2	6.5
B	* Aleutian ♂♂ × Platinum ♀♀	0	0	15	10	6	4.2
C	* Aleutian ♂♂ × Imperial Platinum ♀♀	0	0	17	17	9	3.8
		Dark	Platinum	Aleutian	Sapphire		
D	** Dark (AlalPp) × Dark (AlalPp)	53	17	24	8		
	Expected (9:3:3:1)	57.4	19.1	19.1	6.4		

* Obtained from Associated Fur Farms, New Holstein, Wisconsin.

** Obtained from Messrs. Edward Langenfeld, Boch and Mohr, Andrew Sturgeon and Don Lee.

sapphire color phase is the most nearly "blue" of any of the mutant color phases and for breeding purposes is the most sought type in the history of mink breeding. The data presented in Table IV D show the combined results of *AlalPp* × *AlalPp* matings on four Wisconsin ranches. The observed 53 dark : 17 platinum : 24 aleutian : 8 sapphire is a good fit to the 9:3:3:1 ratio, suggesting that *al* and *p* are borne on different chromosomes ($X^2 = 2.23$, $P > .5$). Sapphires of the two genotypes *alalipip* and *alalpp* are phenotypically indistinguishable and both occasionally have red eyes.

The *Ffalal* genotype is known as the arctic or breath-of-spring aleutian and shows the pattern effect of *F* superimposed on the steel gray of the aleutian. Eric is the aleutian-brown-eyed pastel combination, *alalbb*. This genotype always has pink eyes, darker than the eyes of the albino mink. The pleasing soft tone of the Eric pelage is difficult to describe, but most nearly approaches a bluish fawn color. Individuals of this color phase are often characterized by an irregular splotching, the spots being a few shades further toward the steel gray of the aleutian than the bluish fawn background; factors involved in the splotching are yet to be analyzed.

Green-Eyed Pastel

The green-eyed pastel or golden cross color phase first appeared on the ranch of Mr. Wayne Jones, of Ontario, Wisconsin, in 1941, and so far as is known all green-eyed pastels are descendants of mink from the Jones ranch. The first male and female of this color phase were born along with natural dark kits in litters of two natural dark females bred to the same natural dark male. The male and the females were not known to be related, the two females coming of stock purchased from one ranch and the male from another.

Some green-eyed pastels are considerably lighter in coat color than the average brown-eyed pastel (*bb*). The lightest individuals have lighter brown markings on the underparts which extend upward along the sides of the body, the sides of the neck, and the face; these markings are apparently responsible for the name "golden cross." In the darker individuals the pattern can not be detected, and they appear similar to the brown-eyed pastels. This slight pattern effect may be the result of modifying factors rather than a characteristic effect of the mutant gene per se.

The most characteristic feature of the green-eyed pastel that will usually differentiate it from the brown-eyed pastel phenotypically is the eye color; in most animals the eyes are a luminous green, especially when the mink is in a shaded place looking at the observer. At certain angles in the bright sunlight the eyes appear luminous red, and at other angles are green as seen in the shade. The eye color is occasionally light brown similar to that of the lightest of the brown-eyed pastels; the darker eyed individuals invariably have the darkest pelage, and in general the lighter the coat color, the lighter the eyes. In common with other color phases which have a reduced amount of pigment in the eyes, green-eyed pastels have difficulty in seeing when exposed to bright light, and blink their eyes continuously as if in pain.

Breeders of green-eyed pastels have not reported a ten-

dency toward the nervous reactions called "screw-neck" that is associated with the brown-eyed pastel (*bb*) color phase. A random sample of 48 green-eyed pastels, both male and female, were checked for symptoms of this condition on the Jones ranch in October, 1946; all appeared as well coordinated in their movements as natural dark mink.

TABLE V

BREEDING RESULTS OF THE GREEN-EYED PASTEL COLOR PHASE *

Type Mating	Offspring		Number litters	Average litter size
	Green-Eyed Pastel	Dark		
Green-Eyed Pastel × Green-Eyed Pastel	42	0	14	3.0
Green-Eyed Pastel × Dark (Bgbg)	88	102	48	3.9
Expected (1:1)	95	95		

* Obtained from Mr. Wayne Jones, Ontario, Wisconsin.

The gene (*bg*) for green-eyed pastel behaves as a simple recessive to its non-mutant allele. Data copied from the 1946 breeding records on the Jones ranch are shown in Table V: green-eyed pastel kits only were produced from green-eyed (*bgbg*) × green-eyed (*bgbg*) matings; 48 back cross matings of dark (*Bgbg*) × green-eyed (*bgbg*) gave 88 green-eyed pastels to 102 dark kits, which does not differ significantly from a 1:1 ratio ($X^2 = 1.04$, $P > .30$).

Several breeders have made green-eyed pastel × brown-eyed pastel matings, the resulting kits always being natural dark as would be expected if these color phases result from mutations at different loci. Five individuals which differ phenotypically from either the brown-eyed pastel (*bb*) or the green-eyed pastel (*bgbg*) have been produced on two ranches from *BbBgbg* × *BbBgbg* matings. These pastels are considerably lighter in color than either of the two types of pastel grandparents, and all have red eyes. Although only a small number of these matings have been made, red-eyed pastels which are presumably of the *bbbgbg* genotype have occurred in approximately the proportion expected if *b* and *bg* are on different chromosomes.

Goofus

A pair of dark (wild type) mink produced three very light females in a litter along with dark kits on a small ranch near Winona, Minnesota, " about seven or eight years ago." These three mink appear to be the first representatives of an additional color phase, characterized by a complete lack of pigment in the underfur and a reduction of pigmented guard hairs to approximately one-third the number in the dark mink. The number of pigmented guard hairs varies considerably in different individuals, but the darkest areas are invariably the face, legs and tail in contradistinction to the lighter " points " of the black cross (*Ss*). The lighter individuals of this color phase resemble the Siamese cat.

TABLE VI *

BREEDING RESULTS OF THE GOOFUS COLOR PHASE

Type Mating	Offspring		Number litters	Average litter size
	Goofus	Dark		
Goofus × Goofus	18	0	3	6.0
Goofus × Dark (Oo)	11	7	5	3.6
Expected (1:1)	9	9		

* Obtained from Dr. S. S. Osborn, Waterville, Minnesota.

Dr. S. S. Osborn, who purchased a number of these mink in 1946, has termed this color phase " goofus " for want of a better name. The 1947 breeding results on the Osborn ranch are shown in Table VI. Three goofus × goofus matings produced 18 kits, all goofus. Five matings of goofus to darks having one goofus parent gave 11 goofus to 7 dark, which approximates the 1:1 ratio ($X^2 = .88$, $P = .35$). These limited data suggest that the goofus pattern results from homozygosity for the gene *o*.

SUMMARY

Various workers have reported six mutant genes (*p*, *b*, *S*, *F*, *ip* and c^a) affecting coat color in the ranch-bred mink. In this paper, symbols have been proposed for six additional

genes, and the main effects of these in altering the coat color of the dark (wild type) mink to the royal silver ($s^r s$ or $s^r s^r$), colmira (*Cmcm*), ebony (*Ebeb*), aleutian (*alal*), green-eyed pastel (*bgbg*) and goofus (*oo*) color phases have been discussed. Several color phases resulting from combinations of some of these mutant genes have been described. *S* and s^r are alleles; *Eb* and *b* are linked, being approximately 26 cross over units apart.

The twelve mutant genes can be divided roughly into two groups on the basis of their major effects in changing the wild type (dark) coat color to the different color phases. *F, S,* s^r, *Cm* and *Eb* when singly substituted for their non-mutant alleles alter the natural dark pelage of the mink by producing a pattern. These patterns for the most part result from white spotting (royal silver, black cross), a differential between the pigmentation of the underfur and the guard hair (ebony), or a combination of both (blufrost, colmira). On the other hand, the genes *p, ip, al, b, bg,* and c^r, which produce the recessive color phases platinum, imperial platinum, aleutian, brown-eyed pastel, green-eyed pastel and albino respectively when substituted for their non-mutant alleles, alter the natural dark color by affecting the pigmentation over the entire pelage. The gene for the goofus color phase is the only exception to this rule so far, in that it is recessive to its non-mutant allele, but alters the wild type color by producing a pattern as do the dominant mutant genes.

Acknowledgments

The author wishes to acknowledge the help given and courtesies shown by mink breeders too numerous to mention in allowing use to be made of their extensive breeding records. Special thanks are given to those whose records have been used in this paper. The success of the breeding experiments in the herd at Poynette which have resulted in the data presented is largely due to the sustained interest of Mr. W. A. Ozburn, manager of the Wisconsin Conser-

vation Department's Experimental Game and Fur Farm, and members of his staff, Mr. Kenneth Mills and Mr. Al Thalacker. The writer is indebted to Dr. James F. Crow, Genetics Department, University of Wisconsin, for many helpful suggestions and for reading the manuscript.

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THE MODIFICATION OF THE EXPRESSION OF A POSITION EFFECT[1]

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INTRODUCTION

MANY of the phenotypes in *Drosophila* which are due to chromosomal rearrangements have variable expression, being more or less extreme under different conditions (Demerec and Slizynska, 1937; Kaufmann, 1942). Inversion (2LR)40d is of this type (Hinton and Atwood, 1941). The presence of this inversion in *Drosophila melanogaster* can cause an extreme roughening of the facets of the eye, a mottling of the pigment, and the deposition of black tumor-like material on the surface of the eye. However, there is a great variability of expression, ranging from almost wild-type to an effect so extreme that most of the eye is covered over by black material. A study has been made of the factors involved in the production of this variability in the hope that further understanding may be gained of the phenomenon of position effect and its causes.

Other workers (see below) have described several factors that influence the expression of a position effect, yet there is a paucity of exact quantitative correlations between the expression and the influencing factors. The purpose of the present study has been to secure such a quantitative correlation.

In the study of the mottled white eye, Demerec and Slizynska (1937) were able to demonstrate by selection that genetic factors influenced the expression of the transloca-

[1] A part of this work was carried out at the Marine Biological Laboratory, Woods Hole. A portion of the study was made with the aid of a grant from the American Cancer Society upon the recommendation of the Committee on Growth of the National Research Council.

tion which produced the eye condition. Also, the expression was affected by an extra Y chromosome, by the sex of the individual, and by temperature.

Kaufman (1942) found approximately the same to be true of the roughest inversion. The effect of the Y chromosome was earlier shown in other position effects by Gowen and Gay (1934) and Schultz (1936).

The factors tested in the present set of experiments are sex, temperature, age of individual, age of parent, time of hatching, amount of food, Y chromosome, genetic factors, other chromosomal rearrangements, type of food, and rearrangement of the initial inversion. The last two factors will not be discussed in the present paper but are treated separately elsewhere (Hinton, 1948a, b, and c). Preliminary reports of the effect of some of these factors have been presented (Hinton, 1947 and 1948d).

Material and Methods

All studies were made using *Drosophila melanogaster* heterozygous for Inversion (2LR)40d (Hinton and Atwood, 1941). This inversion is lethal in the homozygous condition. Cytologically one break of the inversion is in the left arm of the second chromosome at 26D (Bridges' map), as seen in the salivary gland chromosomes. The other break of the inversion is in the right arm of the second chromosome at 41A. The break in the left arm is in an euchromatic region; the one in the right arm, in heterochromatin.

The variability of phenotypic expression of the inversion was arbitrarily classified into nine categories as follows:

none (o) = no effect. The eye remains completely wild type in facet arrangement and pigmentation.

barely (b) = a very slight roughening of a few facets. The eye is wild type except for a few displaced facets in the lower edge. The pigmentation is normal.

barely + (b +) = a noticeable roughening of the facets but restricted to several small areas. The eye is otherwise normal.

moderate (m) = the roughening of the facets extends over most of the eye but the displacement of the facets is not

extreme thereby giving the appearance of a moderately rough eye. The pigmentation is normal.

very (v) = the facets are markedly displaced and the pigmentation is mottled, producing some areas lighter or darker than others—there may be a few small black deposits on the surface.

¼ = an extremely rough eye with black deposits, mostly in the ventral-most quarter of the eye.

½ = an extremely rough eye with black deposits extending over the ventral-most half of the eye, with the pigment mottled, as above (v).

¾ = an extremely rough eye with black deposits extending over about the ventral-most three quarters of the eye.

full (f) = the entire surface of the eye is covered with the black deposits which may protrude as irregular mounds or growths from the surface.

All classifications were made by the author in order to lessen the subjectivity of such a scheme of classification as much as possible. Only one eye from each fly was classified; the two eyes are usually affected to the same degree.

Chi-square tests were applied to all comparisons of sets of data. Chi-square values up to 9 (P = .02) are usually not considered in this work as indicating a significant difference. Chi-square values above 12.0 (P = less than .01) are considered as indicating a significant difference. However, in all cases, the general shape of the curve of the plotted data seems to be a more reliable indication of existing differences. This seems to be true because in all cases when two curves are compared, they either lie superimposed upon each other (with minor variations) or overlap only at the extremes. There are few cases where an intermediate condition exists.

Experimental Data

Repeated results. It was found that there was little variability between any two sets of data collected under identical environmental conditions. Fig. 1 shows two populations raised at different times but under identical conditions (in half-pint bottles at 23° C.; 8 pairs of parents of

the same age were allowed to lay eggs for three days). The inversion (2LR)40d was balanced with the Curly inversion (In(2LR)Cy) (Bridges and Brehme, 1944) so all offspring carried both inversions. In the population represented by the solid line, 303 eyes were classified. In the population represented by the broken line, 110 eyes were classified. A Chi-square test gives a Probability of about .65 that two such samples would vary as much as this, or more, at random. Thus, it can be shown that the variability in expression of this phenotype falls within certain limits and that the pattern of variability is maintained under similar environmental conditions.

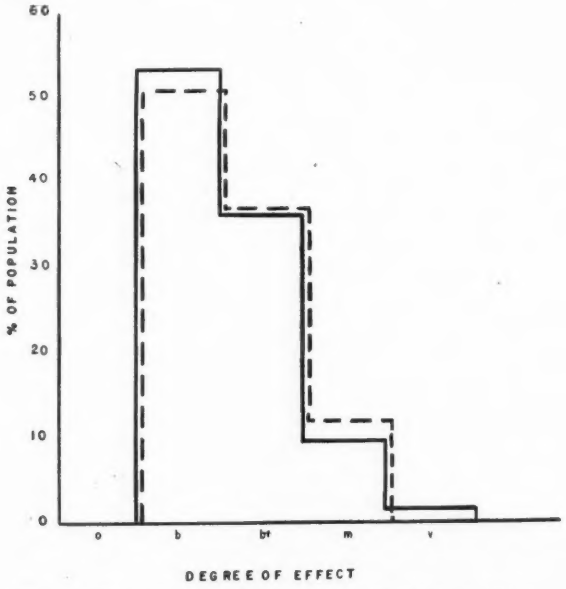

FIG. 1. Two populations raised under identical conditions.

Though the pattern of variability remains the same under similar environmental conditions, that it changes markedly under dissimilar conditions is shown by data which follow. The next step was to define the factors responsible for the

change and to attempt to separate them and observe the effect of each independently, in as quantitative terms as possible.

Influence of sex. To see if any differences in effect dependent upon the sex of the individual existed, males and females were classified separately. Fig. 2 shows a population which was separated according to sex (104 males, represented by the broken line, and 132 females, represented by the solid line). A Chi-square test gives a Probability of .05 to .10 that the two sets of data represent the same distribution (Chi-square = 4.97). It appears from this that the sex of the individual has no effect on the expression of this phenotype.

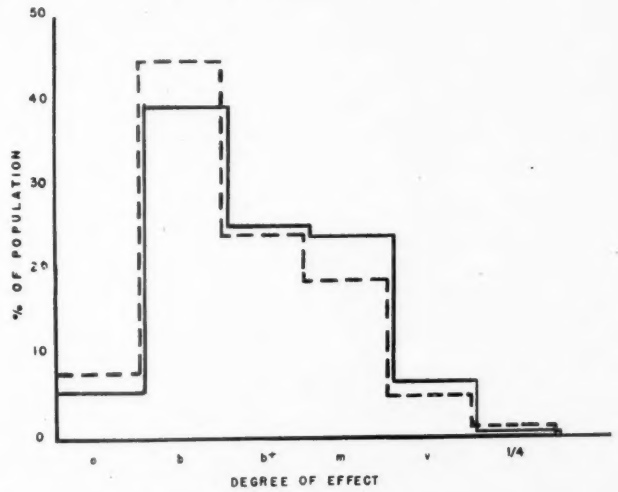

FIG. 2. A population separated according to sex. — — — — — = Males. ———— = Females.

Age of individual. No change in the degree of expression of the phenotype was found in individuals during their adult life. Young individuals were isolated and the degree of effect on the eye noted. When they were examined two weeks later, the degree of effect remained unchanged.

Age of parents. Crosses were made using young or old

males mated to young or old females in all four possible combinations. The same number of parents (8 pairs) was used in each cross. They were given the same amount of culture medium and yeast in half-pint bottles, and all were kept at the same temperature (23° C.); the parents were removed after five days. The offspring were classified and are compared in Fig. 3. From 133 to 222 eyes were classified in each set. Chi-square tests, comparing the sets of data two by two, give Chi-square values of 1.22, 2.90, 5.16, 5.22, 8.84, and 10.10 (P values range from .65 to about .02). Therefore, it appears that there is no significant difference in the phenotype of the offspring whether the parents are young or old.

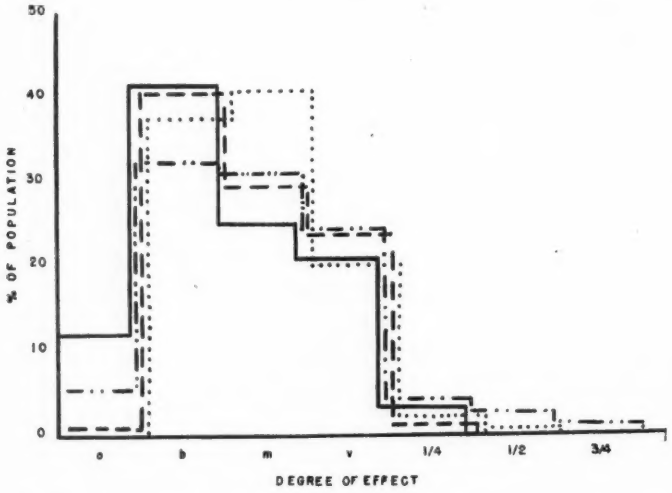

FIG. 3. — — — — — = Old females × old males. — · · — · · = Young females × young males. · · · · · · · · · · = Old females × young males. ———————— = Young females × old males.

Time of hatching. However, a difference in effect is noted when the offspring which emerge from the pupa cases during the first four days are classified separately from those that emerge during the subsequent week. In Fig. 4 four sets of data (taken from Fig. 3) are grouped together

and then divided into two sets according to date of eclosion of the individuals. It can be noted that those offspring which were the last to emerge show a more extreme effect of the eye than do their siblings which hatched earlier. Applying individual Chi-square tests to each of the four sets of data results in Probabilities of less than .01 in three cases and about .03 in the fourth set. [Chi-square values are 12.72 (122 eyes compared to 100), 22.36 (63 compared to 103), 25.55 (67 compared to 56), and 6.30 (63 compared to 123).] These may be considered to be significant differences.

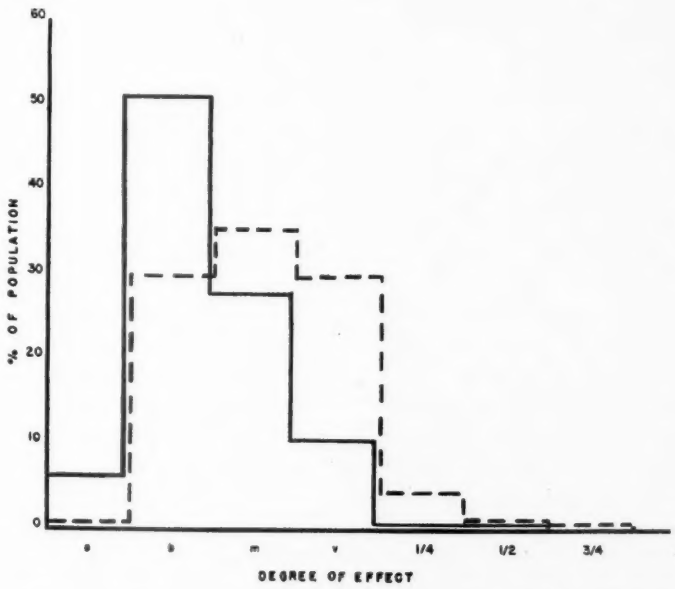

FIG. 4. — — — — — = Last offspring to emerge from the pupa case. ———— = First offspring to emerge.

As a further check on this point, additional data were collected from an experiment more precisely organized. Half-pint milk bottles were used with 6 pairs of parents to a bottle. The parents were allowed to lay eggs for one week and were then transferred to a second set of bottles and

allowed to lay for one week. The time was noted when the offspring started hatching from the pupa case in each set. At the end of one week after the beginning of hatching, the offspring were classified and discarded. Offspring which hatched later than one week after the first hatch, were classified at the end of two weeks. In this way, four sets of data were obtained: the first hatch in the first set of bottles; the later hatch in the first set; the first hatch in the second set of bottles; and the later hatch in the second set.

Such a set-up should enable one to distinguish between the possibility that the increased effect upon the eye in late hatches was due to a depletion of the food supply, and the possibility that the last eggs a female lays are deficient for some factor necessary to the normal eye. The data are presented in Fig. 5. Chi-square tests comparing the four sets of data give the following results: the first counts on each set compared (467 eyes, and 332) give a Probability of less than .01 ($\chi^2 = 12.7$); the second counts on each set compared (561 eyes, and 584) give a Probability of less than .01 ($\chi^2 = 37.05$); the first count compared to the second count in the first set (467 eyes, and 561) gives a Probability of less than .01 ($\chi^2 = 125.05$); the first count compared to the second count in the second set gives a Probability of less than .01 ($\chi^2 = 153.5$); the first count of the second set compared to the last count of the first set gives a Probability of less than .01 ($\chi^2 = 69.8$); and the first count of the first set compared to the last count of the second set gives a still lower Probability ($\chi^2 = 248.8$). It can safely be concluded that there is a significant difference between the first and second counts in each set. Also there is a significant difference between the two first counts, and between the two second counts. Therefore, both the food and the eggs have an influence on the eye.

Crowding. Two types of containers were used in this experiment for raising the flies. One was a five-inch shell vial and the other a half-pint sized bottle. Eight pairs of parents were placed in each type of container. The cultures were raised at 28°. From the vial, 124 offspring were ob-

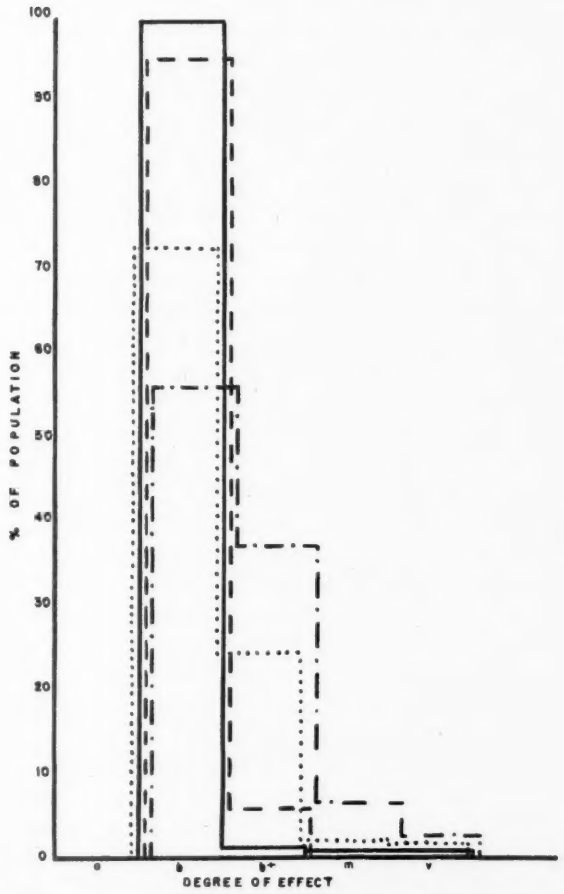

FIG. 5. ———— = First offspring in first bottles. — — — — — = First offspring in second bottles. · · · · · · · · · · = Second offspring in first bottles. · — · — · — · = Second offspring in second bottles.

tained and the eyes classified; from the bottle, 458 offspring. A striking difference in the degree of eye effect in the two sets can be noted by comparing the two distribution curves (Fig. 6). A Chi-square test shows that there is a Probability of less than .01 that these are related sets of data.

The flies raised in the shell vial show much more extreme effect than those raised in the bottle under less crowded conditions.

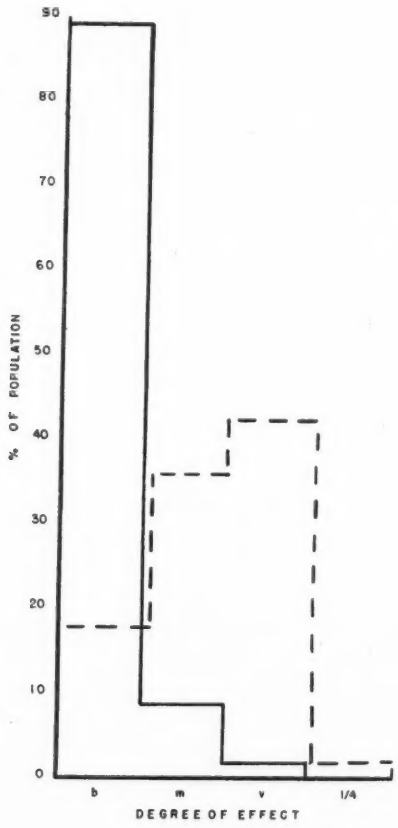

FIG. 6. ———— = Flies raised in half-pint sized bottles. — — — — — = Flies raised in 5-inch shell vials.

Temperature. Culture bottles (half-pint) containing 8 pairs of parents were kept at each of the following temperatures during the entire development of the offspring: 15°, 17°, 19°, 23°, 25°, and 28°. The results of the effect of the

various temperatures on the eyes of the offspring are shown by the distribution curves in Fig. 7. At 15°, there was relatively little expression of the position effect. A statistically significant increase in effect is shown when the temperature is raised by two degrees (P = less than .01, $\chi^2 = 28.25$—186 flies compared to 164). A still greater effect is gained at 19° (P = less than .01, $\chi^2 = 20.41$—186 compared to 101). However, when the temperature is raised to 23°, the curve resembles that obtained at 17° (P = about .20, $\chi^2 = 4.84$—164 compared to 110). At 25° the situation obtained at 15° is somewhat approached (P = about .01, $\chi^2 = 18.45$—186 compared to 272); while at 28°, the picture is almost identical with that obtained at 15° (P = about .75, $\chi^2 = 1.28$—186 compared to 137). It appears that the eye effect is lessened at both extremes of temperature, the expression being most marked at the intermediate temperatures.

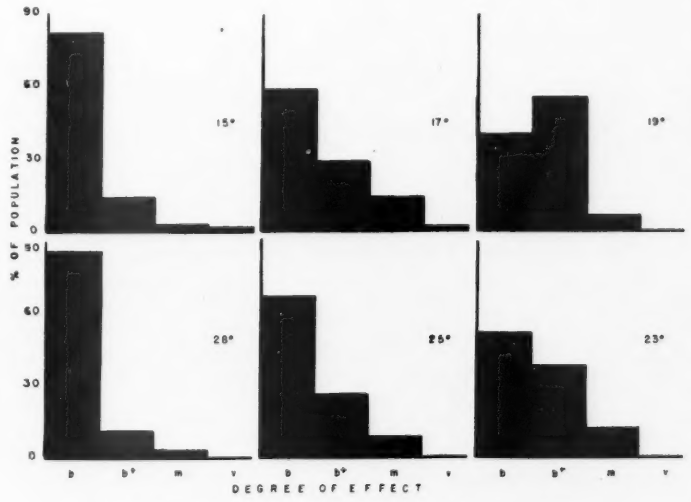

FIG. 7. Populations raised at various temperatures.

Extra Y-chromosome. Flies carrying Inversion (2LR)40d were mated to females known to have "attached" X-chro-

mosomes and a Y-chromosome (±/w). The female offspring (aside from the triple-X "superfemales") would necessarily receive, in addition to the normal number of chromosomes, a Y-chromosome from the male parent. All the male offspring would receive the normal number of chromosomes. A comparison is made in Fig. 8 of the normal male offspring to their sisters carrying the extra Y-chromosome. These data show a significant difference (P = less than .01, $\chi^2 = 49.6$—63 flies compared to 66) in the expression of the inversion. The expression is much less extreme in the case of the females with the Y-chromosome.

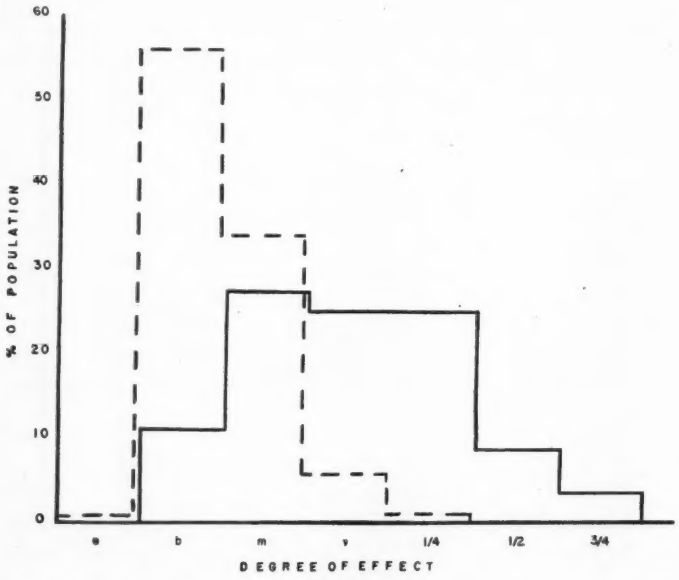

FIG. 8. ———— = Normal male offspring. — — — — — = Sisters with extra Y-chromosomes.

Genetic modifiers. It was noted that in some cases when In(2LR)40d was crossed to another stock of *D. melanogaster,* the resulting offspring showed a more extreme effect from the inversion than the parents had shown. This was

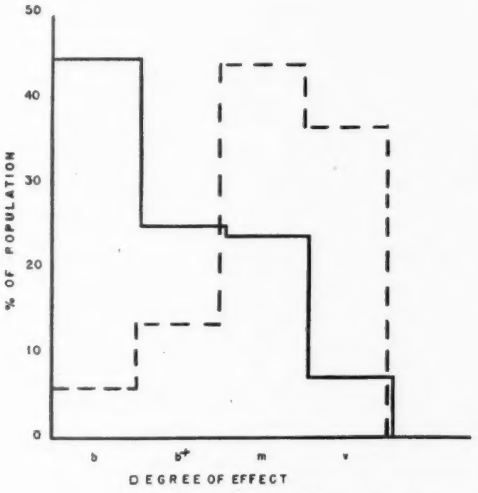

FIG. 9. ———— = Offspring of In(2LR)40d inbred. — — — — = Offspring of In(2LR)40d × Cy/Pm ; H/SbC.

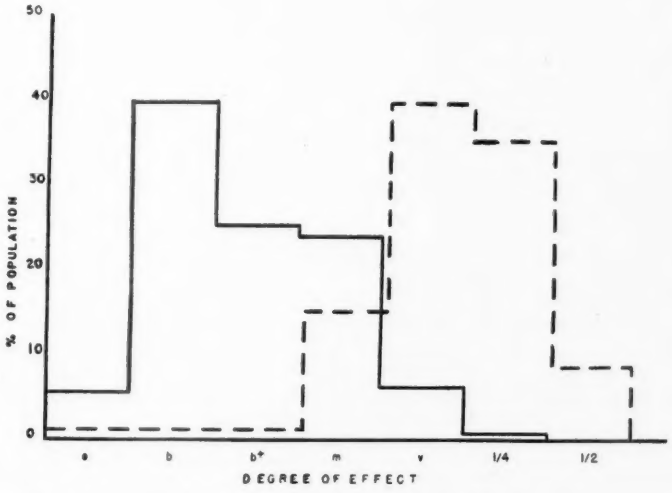

FIG. 10. ———— = Offspring of In(2LR)40d inbred. — — — — = Offspring of In(2LR)40d × Oregon-R wild.

especially true in the cases of crosses with Cy/Pm; H/SbC (Fig. 9) and with Oregon-R wild (Fig. 10). The probabilities of their being samples from the same population are less than .01 in both cases (Fig. 9: $\chi^2 = 31.3$—236 flies compared to 68; Fig. 10: $\chi^2 = 198.7$—236 compared to 98).

However, this is not always the case, since in some outcrosses the phenotype of the offspring remains the same as that of the In (2LR)40d parents regardless of the introduction of genetically different chromosomes. Such is the case when In(2LR)40d is crossed to Bl L^4/Cy (Fig. 11). The Probability that they are the same is about .15 ($\chi^2 = 5.86$—236 flies compared to 111).

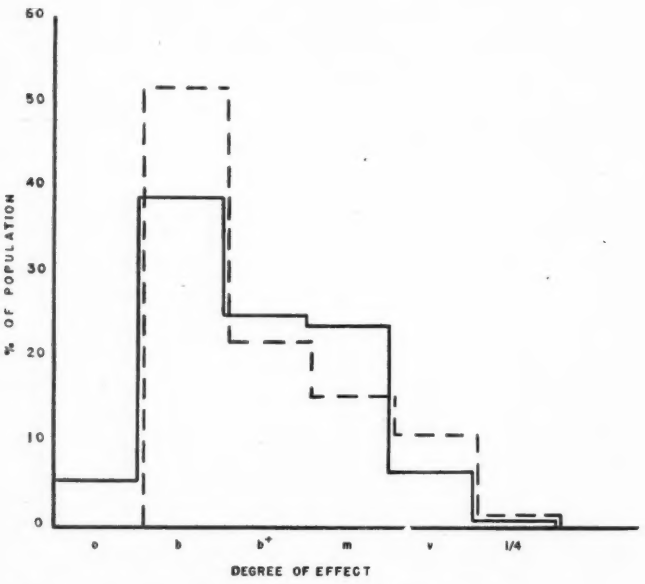

FIG. 11. ———— = Offspring of In(2LR)40d inbred. — — — — — = Offspring of In(2LR)40d × Bl L^4/Cy.

An attempt was made to identify the factors responsible for the change in phenotypic expression which occurred in the offspring of the above-mentioned crosses. For this pur-

pose two series of crosses were carried through. The first of these was a cross intended to produce offspring which would carry all the chromosomes of the In(2LR)40d parent except for the Curly chromosome. This cross is outlined as follows: the wild type chromosomes of the In(2LR)40d parent are represented by an encircled plus sign for convenience in distinguishing them from the wild type chromosomes of other stocks. Each individual is represented by three pairs of chromosomes, the first, second and third, written from left to right in that order. (The small fourth chromosomes are ignored in these crosses.)

TABLE 1

+; Cy/Pm; H/SbC × In(1)dl49/sc ec; +/+; +/+
⊕/⊕; In(2LR)40d/Cy; ⊕/⊕ × In(1)dl49; Cy/+; SbC/+
In(1)dl49/⊕; In(2LR)40d/+; SbC/⊕ × ⊕; In(2LR)40d/+; SbC/⊕
⊕/⊕; In(2LR)40d/+; ⊕/⊕

The phenotype of the final offspring (G) (those identical

FIG. 12. ———— = ⊕/⊕; In2LR/Cy; ⊕/⊕. – – – – – = ⊕/⊕; In2LR/+; ⊕/⊕.

with the In(2LR)40d parents except that they lack the Curly chromosome) were compared to the In(2LR)40d parents (A) shown in Fig. 12. No statistically significant difference exists as indicated by a Probability of .85 (χ^2 = .95—236 offspring in A; 43 offspring in G).

The significance of these results will be dealt with in the discussion.

The other series of crosses is outlined as follows:

TABLE 2

⊕ ; In(2LR)40d/Cy ; ⊕/⊕ × +/+ ; Cy/Pm ; H/SbC
⊕/+ ; In(2LR)40d/Cy ; ⊕/SbC × + ; Cy/Pm ; ⊕/SbC
⊕ or +/+ ; In(2LR)40d/Cy ; ⊕/⊕

The final offspring (F) of this series of crosses were compared to the In(2LR)40d stock (A) as shown in Fig. 13. There is probably no significant difference in the phenotypic expression in the two cases (P = about .20) (χ^2 = 4.76—23 offspring of type F were classified).

FIG. 13. ———— = ⊕/⊕ ; In2LR/Cy ; ⊕/⊕. – – – – – = ⊕/+ ; In2LR/Cy ; ⊕/⊕.

Discussion

In this series of experiments we are dealing with a chromosomal inversion which has a phenotypic expression upon the eye, a position effect. The degree of expression varies from no effect to a very extreme effect depending upon a number of factors. The series of experiments reported here was designed to test the influence of some of these factors upon the degree of expression of the inversion.

From the results, several possible factors can, at the onset, be ruled out; these having little or no influence upon the position effect. These factors are sex, age of the individual, and age of the parents.

The phenotype produced by the inversion is significantly more extreme, however, in crowded cultures. This may be a starvation effect, although the present evidence does not rule out other explanations.

Also to be noted is that the last individuals to emerge from the pupa cases show a more extreme phenotype than do their siblings. This is not due to the age of the parents as has already been shown. It is possible that the last eggs a female lays are deficient for some substance which is necessary for its phenotype to approach the normal. It is also possible that the food source is not as abundant for the later larvae and the extreme phenotype is actually due to a starvation effect just as it was in the case of crowded cultures. Both of these possibilities seem most likely in light of the experiment the results of which are summarized in Fig. 5. In this experiment two factors were noted: the age of the culture medium at the time of hatching, and whether the eggs were among the first or last to be laid by the parents. It appears from the results that the latter of these factors plays a lesser but noticeable role.

For convenience in discussing, the four sets of data are numbered, as follows: (1) first count in first set of bottles; (2) last count in first set of bottles; (3) first count in second set of bottles; (4) last count in second set of bottles.

If the depletion of some ingredient of the food were the

sole factor influencing the increased effect on the eye, it would be expected that counts 1 and 3 would give a similar distribution and that counts 2 and 4 would be similar to each other. If a progressive deficiency in the last eggs of some necessary substance were the sole factor, it would be expected that the effect on the eye would be progressively more severe from count 1 to 4 in the ascending order of 1, 2, 3, 4. However, if both of the above discussed factors were having an influence, it would be expected that the severity of effect would ascend in the order of 1, 3, 2, 4. The Chi-square tests should show a difference between each two sets with less difference between 1 and 3 and an increasing difference in the following order:

1 - 3
2 - 4
3 - 2
1 - 2
3 - 4
1 - 4

When the Chi-square values are arranged in that order, the results are as follows:

12.7
37.05
69.8
125.05
153.5
248.8

The agreement of prediction with results strongly indicates that both factors are influencing the results, namely, that some substance necessary to the normal eye becomes depleted in the food and also in the last eggs laid by the female.

The possibility still remains that some effect is due to a slower development. The influence of the rate of development on mosaics has been indicated by Noujdin (1946). An attempt was made to examine this possibility in the present investigation. Fifty eggs of the same age were placed individually in 50 shell vials with ample medium.

The date of hatching from the pupa case was noted and the eyes examined to see if the last to hatch were more severely affected by the inversion than were the first to hatch. Only 14 of these pupated, and all on the 7th day. They hatched over a period of three days. However, only 3 hatched on the first day and only one on the 3rd, the remaining hatching on the second day. There was not enough difference in the effect on the eye to justify any conclusion; all flies were either barely affected or barely +.

It, therefore, still remains a likely explanation that the influencing factor is the availability of some essential substance. The exact nature of this situation is being tested by raising cultures carrying In(2LR)40d under aseptic conditions on chemically defined medium. The results of these tests will be reported at a later date.

The analysis of the genetic modifiers involved is far from complete, yet certain points appear to be well established. The presence of genetic modifiers seems to be the only interpretation that can be placed upon the results obtained from the crosses, the offspring of which are shown in Figs. 9, 11 and 12.

In the second of these crosses offspring (B) were obtained which differed from the parents (A) in only one respect. That is, one of the wild type X and 3rd chromosomes, and the Curly second chromosome were replaced by chromosomes from the Bl L^4/Cy stock. This apparently had no effect upon the expression of the inversion since these heterozygous offspring show no statistically significant different distribution from the parents. However, when the same parental stock (A) was crossed to Oregon-R wild (D), the offspring (Fig. 10) showed a striking increase in effect. These offspring, like the ones in Fig. 11, had had one wild type and 3rd chromosome replaced by a wild type X and 3rd from another stock. However, they differed in that in these offspring the Curly inversion had been replaced by a wild type second. These two sets of offspring have an identical chromosomal picture except for the lack of the

Curly inversion in the latter set. The only other difference lies in the different origin of the wild type chromosomes involved in the two sets.

The question, therefore, arose as to whether the difference in phenotypic expression was due to the absence of the Cy inversion, or to the genetic constitution of the wild type chromosomes. To help answer this question, a series of crosses was made (as described in Table 1) to obtain offspring (G in Fig. 12) which had all chromosomes from the parental stock (A) with the exception of the Curly inversion. If presence or absence of the Curly inversion is responsible for the change in expression, these offspring should show an extreme effect in Fig. 10. However, as Fig. 12 shows, there is no significant increase in effect and the conclusion is drawn that it is not the absence of the Curly inversion which has an effect but rather the genetic nature of the wild chromosomes. Apparently the Bl L^4/Cy stock carries different modifiers than does the Oregon-R stock, and these modifiers in the two stocks have a different effect on the expression of Inversion (2LR)40d.

There is some indirect evidence to indicate that the modifiers, at least in one case, may be on the third chromosome. It can be noted in Fig. 9 that offspring (C) obtained from a cross of the parental stock (A) to Cy/Pm ; H/SbC show a significant increase in effect, even though the second chromosomes remain identical to those in the parental stock.

A series of crosses (as outlined in Table 2) were made to obtain offspring (F in Fig. 13) with both the second and third pairs of chromosomes identical with the parental stock (A) and only the X-chromosomes being derived from the Cy/Pm ; H/SbC stock. When offspring of that type are compared to the parental stock (Fig. 14) no significant increase is noted. Therefore, it can be concluded that the X-chromosome does not carry modifiers which are capable (at least alone) of enhancing the expression of Inversion (2LR)40d. The effective modifiers, therefore, by process of

elimination, would be supposed to be on the third chromosome. There is no positive evidence that they are on the third chromosome, and there is no evidence to indicate whether the modifier is a single gene or a series of genes.

At both 28° C. and 15° C. the phenotype is close to normal, but is more extreme at intermediate temperatures. There appears to be some optimum temperature at which the inversion can express itself to the extreme. A variation in temperature in either direction from this point causes the effect to be less extreme.

It is always a temptation to search for some basic factor that all the effects have in common. Such a factor is difficult to find in these experiments which show that food supply, temperature, and genetic environment all effect the phenotypic expression of the inversion. However, if the exact nature of the inversion is realized, it at least offers a clue. It should be remembered that the inversion (2LR)40d has one break in heterochromatin and the other in the euchromatic region 26D/E. The resulting configuration is one in which the euchromatic region 26D (distal to the break) and the euchromatic region 26E (proximal to the break) each lie next to heterochromatin. It has been shown (Hinton, 1948a, b and c) that this inversion can produce its characteristic phenotype only when the heterochromatin is in this position. Even rearrangements of the heterochromatin to distances close to, but not adjacent to, region 26D/E cause the phenotype to be normal. Therefore, the position of the heterochromatin appears to be the primary factor involved in this position-effect. If this is true then we might expect any environmental factor which alters the expression of the inversion to alter it by first affecting the heterochromatin.

" U-shaped " curves, like the temperature curve, are not unknown in situations involving heterochromatin. Prokofyeva-Belgovskaya (1945) obtained a similar curve when the amount of heterochromatization in the X-chromosome was studied at temperatures ranging from 14° C. to 30° C.

This recalls the curve, found by Plough (1917) (corrected by Smith, 1936) showing that crossing over is appreciably increased at the two ends of the temperature range. Since it is known that extra Y-chromosomes also increase crossing over (Stern, 1936), there is probably a correlation between heterochromatin and crossing over, and it is possible that the rise in crossing over is due to a temperature effect upon the same heterochromatin mechanism operating in the other cases. This would seem to indicate that heterochromatin is at its maximum efficiency at extreme temperatures if more frequent crossing over and more normal phenotypes may be considered as criteria of a more efficient system. However, it is at the extremes of temperature that Prokofyeva-Belgovskaya finds the least heterochromatization. Therefore, it seems more logical to form an hypothesis based on an inhibitory effect of heterochromatin. It may be assumed that some of the heterochromatin prevents a process basic to such phenomena as crossing over, and that if part of this type of heterochromatin is inserted next to certain normal genes, it may also inhibit their expression (producing a position effect). If this inhibitory effect is enzymatic in nature it would not be surprising to find that, it had an optimum temperature at which it would act, and that at extreme temperatures it would fail to exert its inhibitory effect. That would account for the rise in the frequency of crossing over and the return to the normal phenotype in the present data at the extremes of the temperature range. The fact that extra Y-chromosomes act in the same way as extreme temperatures would seem to indicate that they have the capacity to block the inhibiting action of the other heterochromatin. That the heterochromatin of the Y-chromosome is different from the heterochromatin that we see in salivary gland chromosomes is evidenced by the very fact that the heterochromatin of the Y-chromosome can scarcely be seen at all in salivary gland preparations.

Just as one would not expect all genes to act in the same

way, one would not expect translocated heterochromatin to affect all genes similarly; nor extra Y-chromosomes and temperature to affect all position effects in a like manner. This expectation is borne out by the variety of curves found in the literature describing effects on the expression of position effects (Demerec and Slizynska, 1937; and Stern, MacKnight and Kodani, 1946, as examples). This may be interpreted to mean that the activity of a gene is correlated with its proximity to heterochromatin and that the action of a gene is inhibited if its normal relationship to heterochromatin is altered. However, since some genes seem to be hypomorphic and others hypermorphic, the end result of the inhibition would necessarily be different in different cases.

It could well be supposed that the pairing of the chromosomes is one of the processes which is referred to as being inhibited in the foregoing hypothesis. If pairing were partially inhibited at normal temperatures, crossing over might well be limited as a result. An extreme temperature or an extra Y-chromosome would overcome the effect and allow more exact pairing, thereby increasing the possible amount of crossing over. That pairing is influenced by extra Y-chromosomes is indicated by the observation of Cooper (1948) that pairing in the presence of an extra Y-chromosome was much more complete in the giant neuroblast cells of the brain ganglia of *Drosophila* than it was observed to be by Hinton (1946) when, supposedly, the same type of cells were studied without the extra Y-chromosome. Also this idea would be in agreement with the position effect data if it is assumed that the inserted heterochromatin in some way upsets normal gene behavior by upsetting pairing in that region. This idea has previously been introduced by Ephrussi and Sutton (1944), and Gersh and Ephrussi (1946). By this reasoning, pairing would seem to be dependent upon some basic chemical process (probably enzymatic) involving the heterochromatin of the chromosome.

Unfortunately, the nature of heterochromatin is not suffi-

ciently understood to allow an exact interpretation of the mechanism by which the heterochromatin acts. It is known, however, that heterochromatin differs from euchromatin in its nucleic acid metabolism (Schultz, Caspersson and Aquilonius, 1940). It is conceivable that the factors discussed are, in some way, influencing the nucleic acid metabolism. It would be expected that temperature would have an influence on such a process; that extra Y-chromosomes would; also that starvation or the absence of nucleic acid in the diet would enhance the effect. And it is not too inconceivable that genetic factors might also modify the activity by working through such channels.

Summary

A second chromosome inversion (In(2LR)40d) in *Drosophila melanogaster* causes an effect on the eye of the adult fly. This phenotype is variable in its expression under different conditions. An investigation has been made to determine the relative roles of various factors in the variability of expression. It is concluded that:

(1) If two sets of offspring are raised under identical conditions, the distribution of degree of effect will be the same (Fig. 1).
(2) The sex of the offspring has no effect on the degree of expression (Fig. 2).
(3) The degree of effect does not change with the age of the individual.
(4) The age of the parent is not a factor in determining the degree of effect (Fig. 3).
(5) The last offspring to hatch from the pupa case show a more extreme effect than do the first offspring to hatch (Fig. 4).
(6) Offspring raised in crowded cultures are more extremely affected than offspring raised in cultures with abundant food (Fig. 6).
(7) Offspring from the last eggs laid by the mother are more extremely affected than those from the first eggs (Fig. 5).
(8) Offspring raised at the extremes of the temperature range (15° C. and 28° C.) are less affected than those raised at the intermediate temperatures (Fig. 7).
(9) A Y-chromosome in the female reduces the effect as compared to the normal brothers (Fig. 8).
(10) Genetic modifiers may increase the effect. These are not present in all stocks. In one stock studied, they appear to be on the third chromosome (Figs. 9–13).

It is suggested that all these factors affect the phenotype by working through the heterochromatin and possibly through the nucleic acid metabolism of the region of the chromosome involved in the inversion.

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ON CORRELATION OF THE PHENOMENA ASSOCIATED WITH CHROMOSOMES, FOREIGN PROTEINS AND VIRUSES

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INTRODUCTION

DISCOVERIES of the last decade are casting a new light on the seemingly heterogeneous phenomena associated with chromosomes, foreign proteins, and viruses. Being intrinsically linked with proteins, it is to be anticipated that these phenomena will ultimately be explicated upon the basis of common factors. It has thus seemed desirable to examine them in the light of new information and to attempt to correlate them upon the basis of factors which appear to be basic.

In 1922 Muller suggested that a specific attractive force between homologous chromosomes brings about their pairing during meiosis. These chromosomes may be separated from one another by distances of the order of a micron at the beginning of the pairing process (Fabergé, 1942; Hinton, 1946). For this reason this force is referred to as a *long range* specific attractive force (hereafter referred to as LRSAF), thereby distinguishing it from the short range specific attractive forces which act between molecules at separations of at most a few angströms. In recent years the LRSAF has been the subject of considerable discussion and investigation (*cf.* Bernal, 1940; Bernal and Fankuchen, 1941; Cooper, 1948; Friedrich-Freksa, 1940; Hinton, 1946; Jehle, 1948; Jordan, 1940; Langmuir, 1938; London, 1943; Muller, 1941; Oster, 1947; Pauling and Delbrück, 1940; Vlasow, 1945; Wrinch, 1947). Chromosome behavior pro-

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vides the most convincing indirect evidence for the existence of such a force. In the last few years Rothen (1947, 1948) has obtained direct experimental evidence that "the interaction between large molecules, such as those involved in immunological and enzymatic reactions, may take place through a field of specific long-range forces extending over 200 Å." Although the evidence for a LRSAF is not conclusive, it seems sufficient to warrant their consideration in any discussion of protein-associated phenomena. In the subsequent discussion the existence of such forces has been assumed as a working hypothesis.

If a LRSAF exists between greatly similar and identical protein particles, it is clear that factors which counteract intermolecular attraction should be of particular significance in determining its effects. A primary factor in this category is the coulomb repulsion between protein particles in solution. This repulsion results because of the great electrostatic charges carried by proteins and it is minimized at the pI (isoelectric pH) of the particles concerned. For this reason it is to be anticipated that the pH of protoplasm will influence the effects of long-range attraction. A second factor which tends to prevent the association of attracting particles is their brownian or thermal velocity.

In cases where particle association mediates protein synthesis, the availability of energy and materials from ancillary reactions or directly from the protoplasm itself is of great significance. This is becoming increasingly evident from present-day studies which clearly indicate the prime role played by nucleic acid in protein synthesis.

I. Chromosome Activity

Three fundamental forms of chromosome activity present themselves: synthesis of metabolites, self-duplication, and pairing. The LRSAF is assumed by its proponents to be effective, not only between greatly similar and identical proteins, but also between proteins and their constituent

proteons or *subproteons*.[1] The numerous experiments of Landsteiner (1946) and his collaborators upon the adsorption of haptens by antibodies have shown directly that very simple molecules can be specifically adsorbed by proteins. The essence of well-known hypotheses (*cf.* Alexander and Bridges, 1928; Haldane, 1935; Koltzoff, 1938; Muller, 1922, 1941; Troland, 1917; Wright, 1941, 1945; Wrinch, 1947) is that genes or subgenes specifically adsorb subproteons and then act as a templet guiding the synthesis of these molecules into replica proteins.

The above action appears to be an essential feature of both metabolite synthesis and gene duplication. While this hypothesis of the synthetic process is acceptable it remains to account for the elaboration of the synthesized metabolites for, as Wright (1941) has remarked, the duplication must be associated with a mechanism of separation. The same question naturally arises with regard to the separation features of chromosome disassociation and disjunction. In this respect Darlington (1937) has suggested that changes in the pH of the protoplasm during mitosis and meiosis alter the magnitude of the coulomb repulsion between the similarly charged homologous chromosomes and the centromeres (kinetochores) and centrosomes. Numerous experiments (*cf.* Schrader, 1944) have shown that the chromosomes behave as if negatively charged, and Botta (1932) is convinced that the magnitude of this charge varies with the different phases of cell division. Indirect evidence of changing surface charges on different cell-organs is provided by the changing fixation-staining reaction and the hydration-dehydration and chromosome spiralization

[1] We adopt the terminology of Wrinch (1947) wherein the term *proteon* connotes a native protein unit incapable of division into subunits also having the native protein character. A native protein is then regarded as a proteon or a system of proteons. A *subproteon* is a functional subunit of the proteon chain and might itself be a proteon of a different protein. The term *metabolite* is used in reference to the substances whose synthesis is directly mediated by the chromosomes or by nucleoproteins present elsewhere in the cell.

cycles. Cyclic changes in the surface charge in turn imply a cycle of protoplasmic pH changes (Darlington, 1937; Kuwada, 1939). Thus, for example, the dehydration of previously more dispersed chromatin which renders the chromosomes visible at the end of the resting stage (Darlington, 1937) is believed to be associated with a decrease in the nucleoplasmic pH (Sakamura, 1927). Direct evidence of such changes is afforded by the work of Yamaha (1935), according to which the pH of the nucleoplasm and cytoplasm undergoes cyclic changes during meiosis in *Tradescantia reflexa, Lilium speciosum,* and other plant species. Thus, *varying* coulomb repulsion probably accounts in large part for the mechanics of chromosome separation. To account for metabolite elaboration, however, it is necessary to consider other mechanisms, since this process can occur in cells constantly in a certain mitotic phase, presumably at constant pH, as, for example, the constant mitotic prophases of glandular secretory cells.

Metabolite Synthesis and Elaboration

The elaboration of metabolite molecules after their synthesis might occur automatically as a direct result of factors brought into play by the synthetic process. Since metabolite synthesis results in a replica of the parent templet, the metabolite and the templet will have essentially identical pI's. Thus, they will carry the same charges at non-isoelectric pH's of the nucleoplasm. Perhaps it is the coulomb repulsion to which these charges give rise which is the cause of metabolite elaboration. This repulsion of the metabolite would not be anticipated in the interaction of the templet with the subproteon building blocks used in the synthesis. The subproteons have different pI's from those of the templets to which they are attracted, so that those which carry lesser charges of the same sign or charges of opposite sign could be adsorbed. Moreover, the subproteons may be adsorbed as derivatives, so that, for example, in the absence of free carboxyl groups, ionization of an amino acid derivative as the anion might not occur and this could obviate

coulomb repulsion from the negatively charged chromosomes.

The synthesis and elaboration of metabolites by chromosomes might, then, be visualized as proceeding in the following manner. The LRSAF attracts nucleoplasmic subproteons to the genic templets, where they are adsorbed as specific short-range forces become effective. Under the influence of these templets, and with the required energy available from ancillary reactions or from one of the reactants, the subproteons are synthesized into metabolite molecules. These are immediately ejected from the chromosome as a result of the coulomb repulsion which arises between them and the parent templets.

It is interesting in connection with the above discussion to note the behavior of yeast volutin granules (these are associated with ribose nucleic acid, Brandt, 1941; Caspersson and Brandt, 1940). As the yeast cells enter the growing phase the volutin granules are observed to swell, fragment and gradually disappear in the cytoplasm. This action appears to occur simultaneously with the assumption of synthetic elaborational activity by these granules. It would appear that the volutin granules are aggregates of a large number of identical smaller particles, which repel one another resulting in fragmentation because of the coulomb repulsion which sets in as the cytoplasmic pH leaves the isoelectric zone.[2] After the growing phase of synthesis and elaboration (analogous to metabolite synthesis and elaboration by genes), the pH of the cytoplasm returns to the initial value. The granule particles distributed throughout the cytoplasm, since they now repel one another to a lesser extent, are brought together under the action of the LRSAF, reforming volutin granules. The swelling of the volutin granules before their fragmentation is probably due to their becoming hydrated (hydration-dehydration cycles are

[2] Claude (1943) has observed that the sedimentation rate of the cytoplasmic "small particles" or microsomes is particularly influenced by the pH of the medium.

known to occur in certain cellular components, *cf.* Kuwada, 1937, 1939). This indicates that the pH of the cytoplasm is changing, for, as Fujii (*cf.* Kuwada, 1939) has pointed out, hydration-dehydration cycles must be intimately associated with a changing pH.

Chromosome Duplication and Pairing

It seems unlikely that there should be any fundamental difference between two such closely related phenomena as chromosome duplication and metabolite synthesis. The chromosome duplication process is, perhaps, a mere qualitative modification of this latter process mediated in part by cyclic pH changes. If the change in the nucleoplasmic pH accompanying the advent of the late interphase or the early prophase were in the direction of the pI's of the daughter metabolites and parent templets, the charge upon these particles and the consequent coulomb repulsion between them would be reduced. Then, as the synthesis of metabolite molecules proceeded, they would no longer be ejected from the chromosome but would remain adsorbed at their respective loci of synthesis.

Chromosome Structure and the Role of Nucleic Acid. While we do not have conclusive knowledge of the precise manner of association of the proteins that go to make up the chromosome thread, recent work has been very enlightening. It is known from the work of Mazia and Jaeger (1939), Mazia *et al.* (1941, 1947) and others (*cf.* Catcheside and Holmes, 1947) that the chromosome possesses a continuous protein framework that does not depend upon the presence of nucleic acid. Mazia interprets the results of his enzymatic studies to indicate that the chromosome possesses a fibrous structural or skeletal protein (digestible by trypsin but not by pepsin) which is different from the protein (digestible by pepsin) of which the matrix is composed, and he regards this interpretation to be supported by the ultraviolet absorption measurements of Caspersson (1939). The differential staining and ultraviolet absorption experiments

of Fujii and Yasui (*cf.* Kuwada, 1937) have also differentiated a structural fiber from the matrix. Using the electron microscope, Buchholz (1947) has concluded that a continuous fiber extends the length of the chromosome and that individual genic granules (termed chromomeres[3]) are attached thereto. The recent work of Mirsky and Ris (1947) is especially significant. These investigators extracted isolated thymus lymphocyte chromosomes with 1M NaCl. A fraction of from 90 to 92 per cent. of the chromosome weight consisting largely of desoxyribonucleohistone (identified with basichromatin) was dissolved, leaving behind a coiled thread, the "residual chromosome." Mirsky and Ris concluded that the form of the chromosome is due primarily to the protein thread (definitely non-histone and identified with oxychromatin) of the "residual chromosome," and that the genes are either organized about or form a part of the substance of this structure.

It is well known that large quantities of DNA (desoxyribose nucleic acid) are associated with the chromosomes during mitosis and meiosis. Brachet (1944), using the pyronine-methyl green staining technique, has provided evidence that RNA (ribose nucleic acid) is present in some chromosomes, while Mirsky (1947) and Mirsky and Ris (1947) have shown directly that in liver chromosomes, thymus lymphocyte chromosomes and trout sperm nuclei, varying amounts of RNA are present.

Much recent work shows the intimate association of nucleic acid with protein synthesis (*cf.* Caspersson, 1947; Painter, 1945; Spiegelman and Kamen, 1946, 1947). Caspersson and his collaborators have been especially active in this field, and Caspersson (1947) recently stated that the evidence seems conclusive that in general all protein

[3] It seems desirable to abandon the term "chromomere" since Ris (1945) believes that "chromomeres" are either misinterpreted gyres of the chromonematic helix, points of overlap of chromonemata or heterochromatic sections where the chromonema is more tightly coiled. Ris points out that a gene represents a region of relatively considerable length along the chromonema.

synthesis requires the presence of NA (nucleic acid). Thus, a primary function of the NA appears to be that of participating in the ancillary reactions of protein synthesis and the grosser aspects of chromosome duplication. As a result of studies of the last few years (Astbury and Bell, 1938; Brachet, 1947; Caspersson *et al.*, 1940, 1941, 1942, 1947; Darlington, 1942; Mazia *et al.*, 1939, 1947; Mirsky, 1943; Mitchell, 1943; Painter, 1945; Painter and Taylor, 1942; Pollister and Lavin, 1944; Schultz, 1941; Schultz *et al.*, 1940) a clearer picture of the NA metabolism is emerging. Thus, for example, Brachet (1940) showed that in the fertilized sea urchin egg there is far more RNA than DNA, but that as the egg develops (after fertilization) the DNA content of the nucleus rises and at the same time the quantity of RNA in the cytoplasm falls. This led Brachet to suggest that one type of NA is synthesized at the expense of the other. In this connection, washing a yeast culture removes most of the RNA of the cytoplasm without extracting the DNA from the nucleus, and yet after this treatment the cells grow when placed in a suitable medium (Delaporte, 1939). There is now conclusive evidence that RNA is also present in the nucleolus (Brachet, 1940; Caspersson and Schultz, 1940; Mirsky, 1943), which is known to be very intimately related to the chromosomes and chromosome activity. The present picture of the NA metabolism would appear to be somewhat as follows. The heterochromatin regions of the chromosome (*i.e.*, the nucleolus-associated chromatin) are believed to take part in the synthesis of DNA (and perhaps also RNA) and thus to act as a sort of regulating center for the NA metabolism of the chromosomes and the entire cell. The nucleolar apparatus appears to function in the transfer of NA between the nucleus and the cytoplasm, and to a large extent in the interconversion of the two types of NA. RNA, the only NA normally found in the cytoplasm, takes part in cytoplasmic protein synthesis. Thus, for example, in secreting cells (*cf.* Painter, 1945) there is a continual synthesis of

NA by the heterochromatin. This NA is converted by the nucleolus into products which are transferred to the cytoplasm to yield RNA exclusively. The RNA is in turn consumed in the cytoplasmic processes of synthesis of protein secretory products. Brachet's work shows that the reverse action (*i.e.*, cytoplasm to nucleus) also takes place, so that the NA metabolism may take on a cyclic character.

It seems evident, at any rate, that DNA (and possibly RNA as well) is closely associated with the processes of skeletal unification that serve to unite the synthesized proteins into the continuous chromosome framework. Thus, Schultz (1941) has suggested that a special function of the DNA might be in the actual synthesis of the fibrous proteins of the chromosome, a point of view that he believes to be in accord with the results of the x-ray analysis of Astbury and Bell (1938), and this is essentially the view expressed recently by Caspersson (1947) and Hydén (1947).

There is some evidence that genic duplication may take place more or less independently of the structural fiber–formation process of chromosome reproductive activity. This is provided by observations of the "lamp brush" chromosomes in the oöcytes of many vertebrates (Dodson, 1948; Duryee, 1941; Koltzoff, 1938). These chromosomes are large and extended and appear differentiated into genic granules that stand out sharply like a string of beads. The individual genic granules appear to undergo a series of longitudinal divisions in the egg nucleus, producing lateral branches and loops made up of granules like the original. These branch granules probably act as auxiliary synthetic centers, thereby temporarily increasing the capacity of the chromosomes for metabolite synthesis (*cf.* Dodson, 1948). At a later stage these lateral extensions are lost in the cytoplasm. It would appear that *fundamentally* it is only the lack of the longitudinal continuity of the structural fiber between these branch granules that distinguishes the for-

mation of "lamp brush" chromosomes from chromosome duplication.[4]

The particles which are unified by the formation of the skeletal protein fiber appear to be those genic or subgenic proteins otherwise elaborated as metabolites (or the proteons of these metabolites). The separation of the parent and daughter[5] threads probably occurs somewhat after the manner suggested by Darlington (1937). Thus, changes in the plasm pH may affect an increased coulomb repulsion between the daughter centromeres and a waning of the polar centrosomic repulsion.

The pairing of chromosomes which occurs during meiosis appears to be caused by inherent variations in the nucleoplasmic pH–chromosome pI cycle and the consequent or determining NA attachment-detachment cycle during this process. One of the variations appears to be that during the early prophase the pH of the nucleoplasm and the pI of the chromosomes approximate one another more nearly than during mitosis. The other variation appears as a concomitant temporal one in which the meiotic prophase is initiated before the disassociation of chromatids and the resulting effective saturation of the attractive forces (see below) has taken place. The net result of these and other related variations seems to be that the coulomb repulsion

[4] Painter and Taylor (1942) have pointed out that the granules of the side branches do not contain DNA, while Dodson (1948) has demonstrated the presence of RNA. From the functional point of view these observations are not surprising, since if DNA is chiefly concerned with synthesis of the fibrous proteins of the chromosome, its presence might not be essential in the side branches which apparently originate by duplication of only the mother granules of the chromosome proper.

[5] According to Darlington (1937), observations of the reproductive behavior of ring chromosomes appear to demand that there be an absolute distinction between a parent and daughter thread, rather than a division of the chromosome into two equivalent daughter threads. The synthesis of the daughter thread may occur one mitotic cycle (or more) in advance of the disassociation for evidence is accumulating that the anaphase chromosome consists of 2 or even 4 chromonemata. Thus, the duplicating activity of the chromosome might continue into the anaphase.

between homologous chromosomes is very small during meiotic prophase so that the LRSAF between their genes and centromeres is able to bring them together.[6]

Other Cytological Considerations

Evidence for a Long-Range Specific Attractive Force. The evidence that a LRSAF acts in chromosome mechanics is quite convincing. At mitosis in the diploid of many Diptera (and of numerous other plant and animal species) homologous chromosomes are seen to lie unusually close together on the metaphase plate, with homologous parts lying parallel. The same somatic pairing has been observed between the homologous chromosomes of polyploid plants during mitosis. In this case, with more than two chromosomes being attracted to one another, the groups lie radially instead of parallel. A similar juxtaposition of homologous bivalent and univalent chromosomes (secondary association) has long been known to occur. The smaller, more mobile chromosomes are more likely to show somatic pairing than the larger ones (*cf.* Darlington, 1937; Fabergé, 1942, for a further discussion and references). Thus, a specific long-range attraction (like that which brings the chro-

[6] It is apparent that the LRSAF must be presumed to act to a certain extent between all chromosomes, it being fully effective only in the case of homologues. This follows because of the essentially identical composition of chromatin, the differences, while apparently not great, being highly determinate. Moreover, the structural protein fiber of the chromosome may be of identical composition throughout chromosome complements. Thus, the equilibrium of the metaphase plate may be regarded as resulting (in the plane of the plate) from the equilibrium of interchromosomal forces of attraction and coulomb repulsion. The maintenance of the plate itself seems to reside largely in the strong repulsion between the centromeres and centrosomes. With respect to the above discussion (text) an inverse relationship appears to exist between the extent of association of the chromosome with NA and its negative coulomb charge. In meiosis the chromosomes are associated with greater amounts of NA than during mitosis and they also apparently carry a lesser charge. On the other hand, the centromere, which appears to be highly charged during anaphase movement, is not associated with NA to any noticeable extent (*cf.* Darlington, 1947). This relationship may have significance with respect to the nature of the bonding of the NA to the protein.

mosomes together at meiotic prophase) appears to be acting in somatic pairing and secondary association to modify the even equilibrium of the metaphase plate. Numerous experiments (Hinton, 1946; Muller and Painter, 1929; Van Atta, 1932) have shown that when chromosome aberrations exist, displaced segments tend to pair with their homologues, even though these segments may have been displaced to non-homologous chromosomes. The stretching and pulling out of position thus induced provides unmistakable evidence of this action. From studies of chromosomes undergoing somatic pairing Hinton (1946) concluded that the LRSAF may be effective at distances as great as 10 microns, and for purposes of calculation Fabergé (1942) uses 4 microns as an average distance at which leptotene chromosomes are separated at the beginning of pairing. That the LRSAF which secures chromosome pairing is specific gene for gene is shown by the pairing features in certain cells heterologous with respect to translocations, inversions and other aberrations. In these cells, the pairing of homologous genes occurs even though the chromosomes must be thrown into crosses, loops, etc., in order that this may occur (Belling, 1927; McClintock, 1933; Muller, 1941; Painter, 1934).

Saturation of Attractive Forces. Evidence that the attractive forces become effectively saturated by the association of chromosomes in pairs is provided by observations in several triploid (and tetraploid) plants (Darlington, 1937). On the triploid meiotic cells all three of the homologous chromosomes approach each other in the prophase, but pairing between them appears characteristically between two only in any given region. The third chromosome may be paired with either of the other two at other regions, or if the two are paired throughout their length, the third may remain free altogether, lying parallel to the bivalent. Saturation effects are also shown when chromosomes happen to divide before pairing is completed. In these circumstances the pairing process is brought to a halt, the divided seg-

ments failing to pair with one another (Darlington, 1937).

That the protoplasm does not attain the pI of the chromosomes, even in meiosis, is shown by the fact that there exist at all stages repulsions of various strengths between bivalent and univalent chromosomes. The initial pairing is accomplished because the LRSAF is able to overcome the minimal coulomb repulsion, and not because repulsion has ceased. Thus, after formation of the tetrad, the attractive forces are effectively saturated by the association of the sister chromatids of each dyad, so that the dyads are no longer held together. As a result of the coulomb repulsion these fall apart (in early diplotene), being held together only where chiasmata have formed or at the centromere.

II. Foreign Protein-Induced Phenomena

We turn now to a generalized discussion of the action of foreign proteins. Consider the introduction of a foreign protein (non-nucleoprotein) into the tissues of an organism. Playing no part in the normal metabolism of the organism, the foreign protein would tend to disturb the delicately balanced dynamic equilibrium of the protoplasm. One effect would undoubtedly be the attraction and adsorption of certain subproteons by the foreign protein, in the same manner as do the genes. The synthetic activity of the genes is provided for by the proper ancillary mechanisms which are normally present in the protoplasm. In the case of a foreign protein, however, such ancillary synthetic mechanisms would not be guaranteed. Any synthetic activity of the foreign protein would depend upon the extent to which the normal metabolic processes of the host tissues could be utilized as ancillary synthetic mechanisms for its own duplication. Subproteons of the foreign protein similar to those subproteons normally synthesized by the host tissues in the formation of its own protein complement would be likely to exert synthetic activity. Thus, certain subproteons of the foreign protein (the antigen) could be expected to reproduce themselves, just as in the synthesis of metabolites by the genic templets (the synthetic activity

of these subproteons would be specifically modified by the presence of haptens). If the protoplasmic pH at the site of synthesis were sufficiently distinct from the pI's of the reproducing subproteons (the *antigenic groups*), the synthesized molecules (the antibodies) would be elaborated, after the manner of metabolite elaboration by genes. Thus, the foreign protein would consume normal tissue components and synthesize and elaborate partial replicas of itself. It is unlikely that the organism would possess any adequate *direct* combative mechanism to obviate this derangement of its metabolism; however, the process might tend to check itself in the following way. In other tissues of the organism more isoelectric to the antigenic groups of the antigen, the LRSAF between these groups and the antibodies would be effective and would result in adsorption by the antigen of any of the antibodies encountered there. Thus, in these tissues, the antigen would form *primary complexes* with the antibodies. The increase in size due to this aggregation would result in a decreased brownian velocity of the primary complexes, permitting them to aggregate with one another under the action of the LRSAF to form *secondary complexes*. By this process inert aggregates would be formed. In this regard it is known that the antibodies, wherever they are produced, are rapidly distributed by the bloodstream and are in part fixed by various tissues (Topley and Wilson, 1946). These inert aggregates are probably degraded by the clearing organs or cells (both the macro- and the microphage engulf and digest formed antigenic material). This would automatically tend to prevent the synthetic activity of the antigen from completely disrupting the metabolism of the host.

If an additional amount of the antigen were introduced into the immune organism, the particles thereof would be rapidly inactivated, acting as they would (in certain tissues) as foci of adsorption for the antibodies present. It is presumably the same inactivation process that occurs *in vitro* when antibodies are brought together, under appropri-

ate conditions, with the antigen. It is to be noted that the union of antigen with antibody merely results in inhibition of the synthetic behavior of the antigen through saturation of the LRSAF, and does not in itself act to destroy the properties of either component. Thus, for example, it appears that a more or less loose combination of toxin and antitoxin takes place, the poisonous properties of the toxin being held in abeyance as long as the union persists (Jordan and Burrows, 1942).

A single antigen could give rise to many antibodies, since it merely provides the synthetic templets. The size of the antibody would not necessarily be related to the size of the antigenic group or the antigen, since the antibody need not consist of a single proteon, but might be an aggregate of proteons. These proteons might aggregate (through hydrogen bonding) at the site of synthesis, or they might be brought together through the action of the LRSAF after ejection from the antigenic group. The feasibility of this latter type of action is borne out by Svedberg's (1940) work which shows that soluble proteins are often capable of reversible association and dissociation into particles whose molecular weights are usually some simple multiple or submultiple of that in the original phase. Thus, proteins may be regarded as reversibly dissociable component systems. The work of Svedberg (1940) and his coworkers has also shown that for a given protein the molecular weight as a rule has its maximum value within a given pH range around its pI. Svedberg (1938) concludes that synthesis of the protein particle is probably accomplished by the successive aggregation of definite units.

A certain amount of evidence indicates that antibodies are aggregates of proteons. Thus, in different species immunized, an antigen gives rise to antibodies whose molecular weights vary from 156,000 to 930,000 (Kabat, 1939). Furthermore, the larger antibodies are disaggregated by relatively mild treatment with barium hydroxide without much reduction of precipitating power (Topley and Wilson, 1946), and although antibodies have been found relatively

resistant to the action of pepsin at pH's greater than 3, they undergo partial degradation without loss of antibody function (Schmidt, 1944). In addition, ultrafiltration experiments on horse and rabbit anti-pneumococcus serum have demonstrated the presence of aggregates which are dispersed by changes in the medium (Landsteiner, 1946).

It has been suggested that only those subproteons of the antigen that are closely related to subproteons or proteons of the normal host proteins can act as antigenic groups. This being the case, an essential similarity of all antibodies could be anticipated. Only those subproteons of the antigen greatly similar to proteons of *generally occurring* proteins would be able to manifest antigenic behavior. In this respect, analytical, ultracentrifugal and electrophoretic studies all show highly purified antibodies to be typical proteins, which are very closely related to one or another of the normal serum globulin fractions. A close similarity of antibodies to one another is clearly brought out by experiments in which antibodies themselves are used as antigens in other species (Landsteiner, 1946; Treffers, 1944). Thus, it appears that only those subproteons of the antigen which are closely related to the proteons of the serum globulins may manifest antigenic behavior. This would indicate that the serum globulins possess a rather characteristic distinction, inasmuch as they are pointed up as being similar between different species to the extent that their ancillary synthetic mechanisms are practically interchangeable. Of interest and perhaps significance in this respect is the work of Cannon *et al.* (1945) who fed various foreign proteins to protein-deficient rats. It was found that the best weight recovery and the most effective serum protein regeneration occurred in animals fed certain globulin fractions, while the albumins gave poorest response. These effects are probably related primarily to the content of

essential amino acids[7] of the globulins (Cannon, 1945), but it is possible that globulins (which need not be digested to the amino acid stage) are most readily and efficiently converted to the proteins of the animals to which they are fed.

It has been found (Cohen and Chargaff, 1940) that thromboplastic protein-antibody precipitates are more active in promoting blood clotting than is the antigen contained in them, and that serological inhibition reactions can be obtained with proteoses (*cf.* Landsteiner, 1946). These findings provide rather indirect support for the contention that antibodies consist of proteons identical with certain subproteons of the antigen.

Experimental work has failed to disclose any *consistent* differences in the physical and chemical properties of antibodies and normal serum globulin. Consequently, it must be concluded that the proteons of the antibody and normal serum globulin, while differing serologically, are otherwise greatly similar. This serological distinction probably resides in differences in the composition and spatial distribution or configuration of antibody subproteons from those of normal serum globulin. Breinl and Haurowitz (1930) and Mudd (1932) have suggested that the ordering of the amino acids in the polypeptide chain of the antibody might be different. On the other hand, Rothen and Landsteiner (1939) pointed out that different ways of folding the same polypeptide chain might be involved, and this is essentially the view adopted by Pauling (1940). Practically the same situation is found to occur in the case of the highly potent soluble bacterial toxins, which do not seem to differ in any essential respect from bland proteins. The balance of evidence indi-

[7] The essential role of the serum globulins is unknown but in view of their high content of essential amino acids and our conclusion that they are probably greatly similar between different species, the possibility suggests itself that they bear somewhat the same relationship to the amino acids of the blood as hemoglobin does to oxygen. Thus, the globulins and amino acids may form loose reversibly dissociable adsorption complexes, the equilibrium being governed by variations in the plasma characteristics induced by the state of the specific tissues encountered.

cates that the toxicity is a property residing in the structure of the toxin molecules (Jordan and Burrows, 1942). In any case, the different behavior of normal and immune globulin toward the antigen would be accounted for, since the LRSAF appears to be highly dependent upon specificity of structure.

For the sake of simplicity the importance of the pH in the antigen-antibody aggregation process has been stressed. It is to be noted, however, that agglutination and precipitation *in vitro* usually do not occur in the absence of electrolytes. Yet it is only the macroscopic effect (formation of secondary complexes) that is lacking, for in both cases it has been shown (*cf.* Landsteiner, 1946; Topley and Wilson, 1946) that the antigen-antibody union (formation of primary complexes) takes place. This latter phenomenon serves to emphasize the real distinction between the initial formation of primary complexes and further aggregation leading to precipitation. The electrolytes appear to reduce the charge carried by the interacting particles, thereby reducing the pH sensitivity. It is known (Coulter, 1921; von Euler and Brunius, 1931) that pH changes affect the adsorption of antibody by the antigen, especially in the absence of electrolytes, although there appears to have been no systematic investigation of this factor. The above considerations of the antigen-antibody reaction have been patterned primarily after the aggregation of antigen with precipitins or antitoxins, where the brownian velocity is probably a significant factor.[8]

[8] In the precipitin reaction, for example, the rate of precipitation is increased with an increase in temperature. However, the concomitant increase in the velocity of the particles reduces the probability that aggregation will occur and decreases the stability of the aggregates, so that beyond a certain temperature, dissociation of the antigen-antibody complex occurs. The formation of primary complexes is evidently the factor that brings the brownian velocity of the antigen down to the effective secondary aggregation zone, while increases in the temperature to values that do not exceed the upper limit for this zone can further enhance the flocculation.

(*To be continued*)